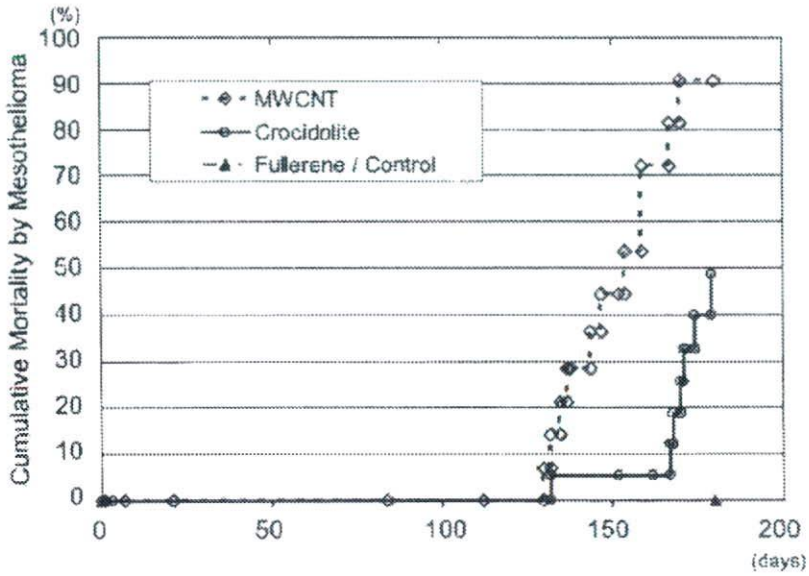

Fig. 8. Cumulative mortality of MWCNT and crocidolite treated mice by mesothelioma: Mice with large/invasive mesotheliomas considered as cause of death are plotted by Kaplan-Meier method. Second major cause of death was constriction ileus due to severe peritoneal adhesion. Among those moribund/dead or terminated at week 25 (day 180), there were 3 mice with incidental mesotheliomas in the MWCNT group and 6 incidental mesotheliomas in the Crocidolite group. No tumor induction was observed in the Fullerene and the Control groups.

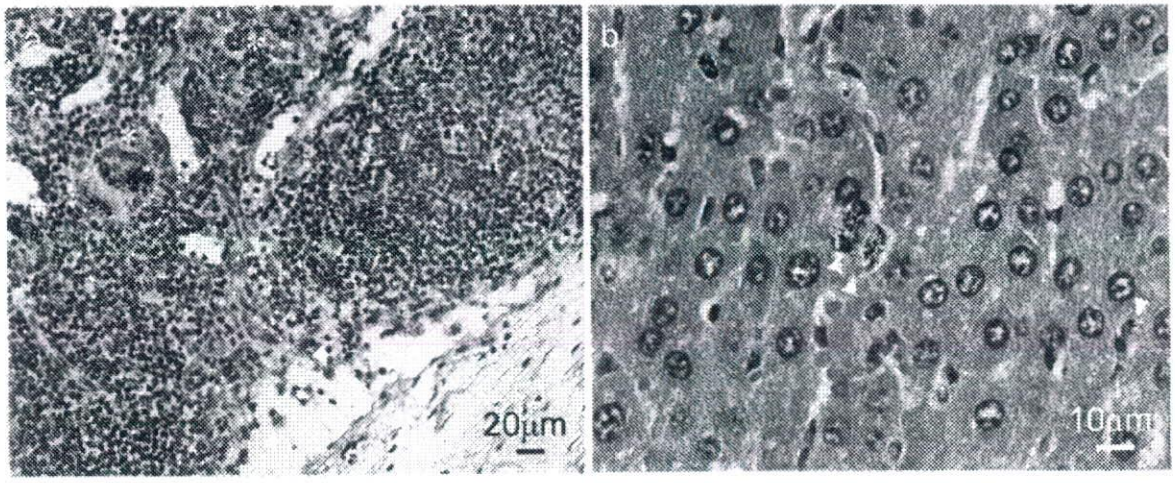

Fig. 9. Extraperitoneal migration of shorter fibers:
Phagocytized shorter fibers are found in hepatic sinusoids and local lymph nodes. a) Multinuclear giant cells (asterisks) and mononuclear phagocytic cells (arrow heads) with black fibers are seen in mesenteric lymph nodes (MWCNT-treated moribund mouse on day 159 with mesotheliomas and fibrous adhesion). b) MWCNT-laden phagocytic cells in hepatic sinusoids (arrow heads)(MWCNT-treated mouse found dead on day 84 with multiple mesotheliomas up to 0.7×0.7 cm in size, severe peritoneal fibrosis and pleural effusion).

tant to the solvents.

In summary, intraperitoneally administered MWCNT has induced mesothelioma in the p53(+/−) mice carcinogenesis model, probably due to its resemblance to asbestos in size and shape, and biopersistency.

DISCUSSION

The foreign body carcinogenesis is a category among various mechanisms of carcinogenesis. It has been postulated that ROS and/or RNS generated locally by the inflammatory reactions against non-digestive, long-lasting foreign bodies induces carcinogenic response (Tazawa *et al.*,2007). And one particular shape and size to enhance

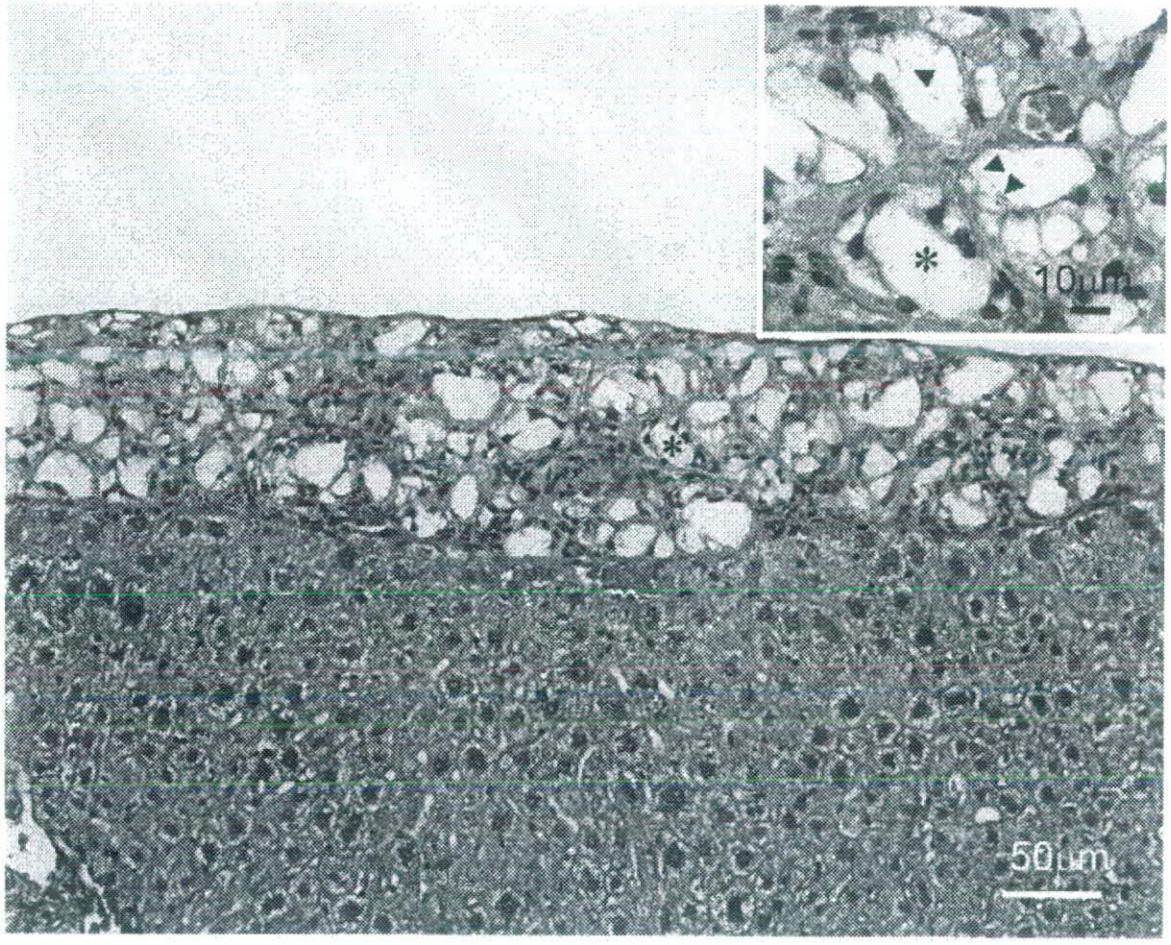

Fig. 10. Fullerene deposits:
Serosa of fullerene-treated mice showed minimum response within this 25-week period. Only black spots were occasionally seen on the surface. Histologically, the spots were made of polygonal slits surrounded by foamy cells and fibrous septa forming a compact fibrous scar. There were no signs of mesothelial response by this treatment. Since fullerene dissolves well to organic solvents especially xylene, the embedded particles were washed away leaving clefts behind. It is noted that the edges of the clefts are tinted brown, indicating possible biodegradation of the surface of the fullerene particles by the phagocytic cells, blending proteins and/or other organic components so that the sub-micrometer fullerene grains become resistant to the solvents (arrow head in inset)

this potency has been extensively studied on asbestos and man-made fibers (WHO, 1986, 1998). To study the asbestos-type carcinogenesis, the intraperitoneal route has been adopted in parallel to inhalation or transtracheal route of lung exposure. There has been some debate on whether rodent models are equivalent to the inhalation exposure to humans (Pott *et al.*,1994). Current understanding would be that the intraperitoneal model has considerable value on hazard identification in this regard (WHO, 1998, 2002). On the other hand, the p53 (+/–) mice, in general, have been suggested to be a good model to predict carcinogen, especially of a genotoxic nature (Pritchard *et al.*,2003). Relatively recently, this model has been reported to be sensitive to oxidative stress-mediated carcinogenesis such as foreign body carcinogenesis, producing a tumor with shorter latency periods than in wild-type mice (Tazawa *et al.*,2007). When asbestos was applied intraperitoneally to this model, mesotheliomas were induced with short latency as well (Marsella *et al.*,1997; Vaslet *et al.*,2002). Here, although the genotoxic effect of MWCNT is unclear, our results suggested that intraperitoneal administration of MWCNT possesses carcinogenic potential in p53(+/–) mice presumably depending on its size/shape and persistency in the organism.

Prediction of the mesotheliomagenic potential of MWCNT in humans cannot be completed by this p53+/– mouse model study. For example, glass fiber of a same shape and size to asbestos tends to fail to induce mesothelioma in humans because of its relatively faster disappearance from the deposition sites (Lippmann, 1990). Biodurability of MWCNT has to be rigorously tested before making any strong regulatory action. Likewise, Fe content of the material may be an important aspect to its carcinogenicity although our MWCNT contained lower Fe than crocidolite (WHO, 1986).

As shown in Fig.1, MWCNT studied here consists of rods and fibers of various size. In general, a bulk of a nanomaterial may contain a wide spectrum of particles at least in their size, from tens of micrometer down to true nanometer ranges. As suggested in this study by fullerene, micrometer-sized particles may become much smaller by biological activities, such as foreign body digestion activities of phagocytic cells. And yet, it is important to limit the significance of this study to the monitoring of biological activity of a compartment of the MWCNT longer than 5 micrometer. There is no information that this study method would be sensitive to pure nanometer-sized particles within this timeframe, i.e. 25 weeks. Again, this study is considered sufficient for detection of mesotheliomagenesis only by rod-shaped micrometer-sized particles. The biological effects of pure nanometer-sized CNTs and fullerene are not assessed in this study, and therefore, this remains open to further research.

The safety assessment for the new materials such as nanoparticles poses a new paradigm. The key to it is that the full-scale exposure to the public has not yet started. Therefore, there is a good chance that the information from hazard identification studies can directly be fed back to the product development plans so that harmful exposure can be prevented before it happens. In this way, manufacturers can produce safer products without risking themselves and the consumers by waiting for the full chronic toxicology studies including carcinogenicity studies to be finished after their initial (less safe) products are widely marketed.

ACKNOWLEDGMENTS

The authors thank Mr. Masaki Tsuji for technical support. This study was supported by Health Sciences Research Grants H17-kagaku-012 and H18-kagaku-ippan-007 from the Ministry of Health, Labour and Welfare, Japan.

REFERENCES

Bernstein, D.M. and Riego Sintes, J.M. (1999): Methods for the determination of the hazardous properties for human health of man made mineral fibres (MMMF). In: European Commission Joint Research Centre. Institute for Health and Consumer Protection, Unit: Toxicology and Chemical Substances. European Chemicals Bureau. pp. 44-45.

Coussens, L.M. and Werb, Z. (2002): Inflammation and cancer. Nature, **420**, 860-867.

Donaldson, K., Aitken, R., Tran, L., Stone, V., Duffin, R., Forrest, G. and Alexander, A. (2006): Carbon nanotubes: A review of their properties in relation to pulmonary toxicology and workplace safety. Toxicol. Sci., **92**, 5-22.

Gulumian, M. and van Wyk, J.A. (1987): Hydroxyl radical production in the presence of fibres by a Fenton-type reaction. Chem. Biol. Interact., **62**, 89-97.

Hei, T.K., Xu, A., Huang, S.X. and Zhao, Y. (2006): Mechanism of fiber carcinogenesis: From reactive radical species to silencing of the beta igH3 gene. Inhal.Toxicol., **18**, 985-990.

Hou, P.-X., Xu, S.-T., Ying, Z., Yang, Q.-H., Liu, C. and Cheng, H.-M. (2003): Hydrogen adsorption/desorption behavior of multi-walled carbon nanotubes with different diameters. Carbon, **41**, 2471-2476.

Jiang, L., Zhong, Y., Akatsuka, S., Liu, Y.T., Dutta, K.K., Lee, W.H., Onuki, J., Masumura, K., Nohmi, T. and Toyokuni, S. (2006): Deletion and single nucleotide substitution at G:C in the kidney of gpt delta transgenic mice after ferric nitrilotriacetate treatment. Cancer Sci., **97**, 1159-1167.

Lam, C.W., James, J.T., McCluskey, R., Arepalli, S. and Hunter, R.L. (2006): A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks. Crit. Rev. Toxicol., **36**, 189-217.

Lippmann, M. (1990): Effects of fiber characteristics on lung deposition, retention, and disease. Environ. Health Perspect., **88**, 311-317.

Luo, J., Peng, L.-M., Xue, Z.Q. and Wu, J.L. (2004): Positive electron affinity of fullerenes: Its effect and origin. J. Chem. Phys., **120**, 7998-8001.

Marsella, J.M., Liu, B.L., Vaslet, C.A. and Kane, A.B. (1997): Susceptibility of p53-deficient mice to induction of mesothelioma by crocidolite asbestos fibers. Environ. Health Perspect., **105** (Suppl. 5), 1069-1072.

Moalli, P.A., MacDonald, J.L., Goodglick, L.A. and Kane, A.B. (1987): Acute injury and regeneration of the mesothelium in response to asbestos fibers. Am. J. Pathol., **128**, 426-445.

Pott, F., Roller, M., Kamino, K. and Bellmann, B. (1994): Significance of durability of mineral fibers for their toxicity and carcinogenic potency in the abdominal cavity of rats in comparison with the low sensitivity of inhalation studies. Environ. Health Perspect., **102** (Suppl. 5), 145-150.

Pritchard, J.B., French, J.E., Davis, B.J. and Haseman, J.K. (2003): The role of transgenic mouse models in carcinogen identification. Environ. Health Perspect., **111**, 444-454.

Roller, M., Pott, F., Kamino, K., Althoff, G.H. and Bellmann, B. (1997): Dose-response relationship of fibrous dusts in intraperitoneal studies. Environ. Health Perspect., **105** (Suppl. 5), 1253-1256.

Tazawa, H., Tatemichi, M., Sawa, T., Gilibert, I., Ma, N., Hiraku, Y., Donehower, L.A., Ohgaki, H., Kawanishi, S. and Ohshima, H. (2007): Oxidative and nitrative stress caused by subcutaneous implantation of a foreign body accelerates sarcoma development in Trp53+/– mice. Carcinogenesis, **28**, 191-198.

Tsukada, T., Tomooka, Y., Takai, S., Ueda, Y., Nishikawa, S-I., Yagi, T., Tokunaga, T., Takeda, N., Suda, Y., Abe, S., Matsuo, I., Ikawa, Y. and Aizawa, S-I. (1993): Enhanced proliferative potential in culture of cells from p53-deficeint mice. Oncogene, **8**, 3313-3322.

Vaslet, C.A., Messier, N.J. and Kane, A.B. (2002): Accelerated progression of asbestos-induced mesotheliomas in heterozygous p53+/– mice. Toxicol. Sci., **68**, 331-338.

World Health Organization (1986): Environmental Health Criteria, **53**. Asbestos and Other Natural Mineral Fibres. World Health Organization, Geneva.

World Health Organization (1998): Environmental Health Criteria, **203**. Chrysotile Asbestos. World Health Organization, Geneva.

World Health Organization (2002): Man-made vitreous fibres. IARC monographs on the evaluation of carcinogenic risk to humans., Vol., **81**, IARC Lyon.

YAKUGAKU ZASSHI 128(12) 1727—1732 (2008) © 2008 The Pharmaceutical Society of Japan

—Reviews—

ナノマテリアルの有害性評価（毒性評価）

津田洋幸,[a] 徳永裕司,[b] 広瀬明彦,[c] 菅野　純*,[d]

Hazard Identification of Nanomaterials

Hiroyuki TSUDA,[a] Hiroshi TOKUNAGA,[b] Akihiko HIROSE,[c] and Jun KANNO*,[d]

[a]*Department of Molecular Toxicology, Graduate School of Medical Sciences, Nagoya City University, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan,* [b]*Division of Environmental Chemistry,* [c]*Division of Risk Assessment, Biological Safety Research Center, and* [d]*Division of Cellular and Molecular Toxicology, Biological Safety Research Center, National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagayaku, Tokyo 158-8501, Japan*

(Received July 15, 2008)

It is considered that the materials with new properties may lead to novel biological effects or unknown adverse health effects. To gather proper hazard information, it is important to develop both experimental protocols and detection/measurement methods for nanomaterials in the body, in parallel. Since 2005, we are running research projects to develop methods to monitor health risk effects for the assessment of manufactured nanomaterials funded by the Ministry of Health, Labour and Welfare. For the experimental protocols, these projects focus on the development of 1) *in vitro* experimental systems, 2) *in vivo* experimental systems (mainly focusing on long-term health implication, especially carcinogenesis), and 3) proper inhalation system. Firstly, fullerene (C60), titanium dioxide and multi-walled carbon nanotube were chosen to be tested because of their high production volume. Safety issues for new materials such as nanoparticles is a new paradigm. The key is that the full scale exposure to the public has not been started yet. Therefore, there is a good chance that information from hazard identification studies can be directly fed back to the product development plan. Manufacturers can produce safer products without risking themselves waiting for the toxicology studies to be finished after their products are widely marketed.

Key words——toxicity; titanium dioxide, multi-wall carbon nanotube (MWCNT); asbestos; fullerene; Absorption, Digestion, Metabolism and Exertion (ADME); carcinogenesis

ナノマテリアルは，今後の産業の新たな発展にとっての重要な新素材である．これらは，従来の素材とは異なった様々な特性を有していること，及びその有害性についても新しい問題が提起されるとの予測がなり立つことから，国民の関心を呼んでいる．しかし，これらの有害性についての研究が始まったばかりであることから，情報は少ない．Figure 1に，最近の動向を示す．2004年に産業技術総合研究所が公開フォーラムを開催し，2005年初頭に経済産業・文部科学・環境・厚生労働の4省庁関連研究所と産学が集まり「ナノテクノロジーと社会」(http://www.aist.go.jp/aist_j/research/honkaku/symposium/nanotech_society/050201/sdata.html)を開催した．これが，しばらくの間，関係各機関の連絡の場となった．

Figure 2に，現在進行中のプロジェクトを示す．厚生労働省は厚生労働科学研究費補助金（化学物質リスク研究事業）を基に，国立医薬品食品衛生研究所を中心とした研究を開始した．Figure 3に示すごとく，2004年より系統的な研究を行っている．意図的に生産されるナノマテリアルについて，人及び環境に対する有害影響の同定（有害性評価）のための研究を開始した．2004年の情報収集と研究方針出しに続き，2005年からのフラーレンや二酸化チ

[a]名古屋市立大学大学院医学研究科・分子毒性学分野（分子医学講座生体防御・総合医学専攻）（〒467-8601 名古屋市瑞穂区瑞穂町字川澄 1），[b]国立医薬品食品衛生研究所環境衛生化学部（〒158-8501 東京都世田谷区上用賀 1-18-1），現，医薬品医療機器総合機構品質管理部基準課（〒100-0013 東京都千代田区霞が関 3-3-2），[c]国立医薬品食品衛生研究所安全性生物試験研究センター総合評価研究室，[d]同毒性部（〒158-8501 東京都世田谷区上用賀 1-18-1）
*e-mail: kanno@nihs.go.jp

本総説は，日本薬学会第128年会シンポジウム S32で発表したものを中心に記述したものである．

- In 2004, the Technology Information Department of AIST launched an open forum entitled "***Nanotechnology and Society***."
- The first symposium (Feb. 2005) was organized by the four national research institutes with four different ministries.

National Institute of Advanced Industrial Science and Technology, **AIST**
National Institute for Materials Science, **NIMS**
National Institute for Environmental Studies, **NIES**
National Institute of Health Sciences, **NIHS**

Importance of multidiscipline network

- In 2005, the survey project of "**Research Project on Facilitation of Public Acceptance of Nanotechnology**" was conducted by the above four institutes and universities. (funded by MEXT)

➡ **Political Proposals**

In 2006, the second project of "The multidisciplinary experts panel for nanotechnology implication"
(MEXT: Ministry of Education, Culture, Sports, Science and Technology)

Fig. 1. Importance of Multidiscipline Network

Research programmes or strategies designed to address human health and/ or environmental safety aspects of nanomaterials;

- Ministry of Economy, Trade and Industry : (2006-2010) project
  The **NEDO** project: "Evaluation of the Potential Risks of Manufactured Nanomaterials based on Toxicity Tests with Precise Characterization."
  The project focuses on toxicity test protocols (mainly an inhalation test) and a risk assessment methodology of manufactured nanomaterials.
- Ministry of the Environment : National Institute of Environmental Sciences (**NIES**) has started a nanotoxicology programme
  - interaction of nano-fibers including CNT with cell membranes,
  - tamasepithelial and transpulmonary migration of manoparticles,
  - *in vitro* and *in vivo* toxicity assay of manomaterials

  NIES has been investigating inhalation effects of atmospheric nanoparticlesfor the last 3 years
- Ministry of Health, Labour and Welfare :The National Institution of Occupational Safety and Health (**JNIOSH**) will start a new research on possible health issues in April 2007, due to exposure to nanomaterials in the workplace
- MHLW the Office of Chemical Safety **NIHS**

Fig. 2. Research Programmes and Strategies Designed to Address Human Health and/ or Environmental Safety Aspects of Nanomaterials

Recent research activities in NIHS granted by the "Health and Labour Sciences Research Grants" of the Office of Chemical Safety, MHLW
(Ministry of Health, Labour and Welfare)

Fiscal year	
2004	Survey research of public information about health implication of nanomaterials
2005	The initial research on methodology of health risk assessment of manufactured nanomaterials started
2006 (to 2008)	Restating the project of "Research on the hazard characterization and toxico-kinetic analysis of manufactured nanomaterials for the establishment of health risk assessment methodology", as the expanded project.
2007 (to 2009)	"Research on the dermal toxicity evaluation methodology of the manufactured nanomaterials"

Fig. 3. Recent Research Activities in NIHS Granted by the "Health and Labour Sciences Research Grants" of the Office of Chemical Safety, MHLW

タンを例とした測定法と有害性評価法の検討，2006年からのMWCNTを加えた長期毒性とトキシコキネティクス，ADME検討法の確立，*in vitro* 法の検討，さらに，2007年からの皮膚毒性，経皮毒性，経気道毒性を加えて，長期毒性に焦点を当てた総合的な有害性評価を開始している．

現在，手元にある意図的生産品としてのナノマテリアルは，凝集体として，あるいは，それ固有のサイズ分布の広範性により（サイズによる厳密な分画が行われていないバルク剤），マイクロメーターの次元を持つ粒子から，ナノメーターの次元を持つ粒子までが混在したものが一般的である．フラーレンはその溶媒の条件により，数百マイクロメーター大の粒子から，完全な単分子状態，さらに，溶解条件の変動により，石英状の柱状-アスベスト様の棒状の凝集体（ウィスカー）に再凝集する性質がある．このような，溶解環境の変化は，生体内では親水性（気管・気管支内，内消化管内，血液内），親油性（細胞膜など，脂質二重膜内），あるいは酸化的環境（炎症細胞による攻撃），その他，酵素的な環境を含め，複雑であることから，Fig. 4に示すごとく，体内への吸収と分布，蓄積と再分解，再分布といった複雑な動態が予測される．蓄積場所についても，血行性，リンパ行性といった移動媒体を使って脳をはじめとする全身臓器に運ばれることが想定され，または報告されている．主な排出経路としては腸管と腎が想定されるが，胆汁への移行，腎糸球体の基底膜親和性・透過性など，不明なことが多い．

実験に用いたMWCNTについては，東京都健康安全研究センターとの共同で電子顕微鏡によるサイズ，及びICP-MS等による金属等の元素含有量の測定を行った（Fig. 5）．径は約100 nmが中心で，長さは5 μm以上のものが約1/4を占めているMWCNTであった．鉄の含有量は3,500 ppmであり，以下ハロゲンや硫黄が認められた．

In vivo 毒性試験として，いくつかの試みを実施しているので，それらについて簡単に述べる．まず二酸化チタンの皮膚発がんプロモーション作用について，ヒトc-Ha-ras導入ラットを用いた検討を紹介する（Fig. 6）．ジメチルベンツアントラセン（DMBA）を1回塗布し，30週間，二酸化チタンを塗布した．陽性対象には皮膚発がん促進作用の知られるフォルボールエステル（TPA）[1]を，陰性対象

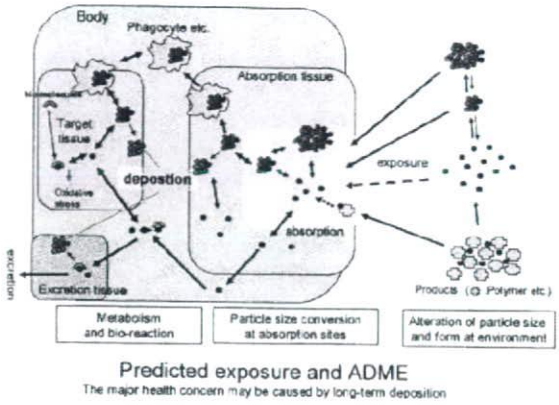

Fig. 4. Predicted Exposure and ADME

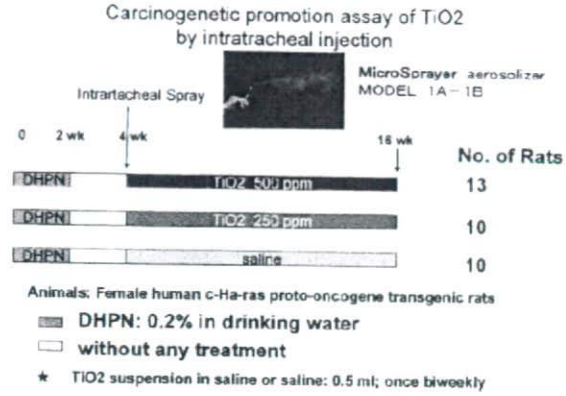

Fig. 7. Carcinogenetic Promotion Assay of TiO_2 by Intratracheal Injection

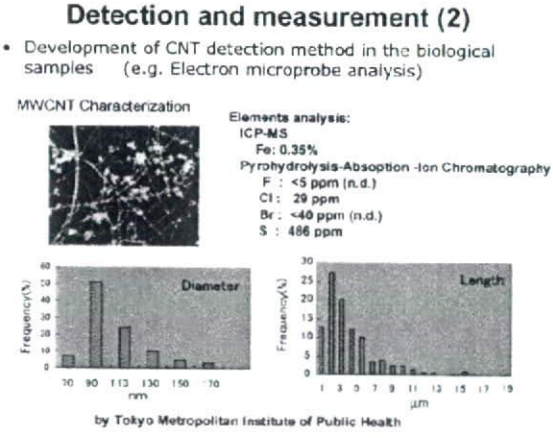

Fig. 5. Development of CNT Detection Method in the Biological Samples

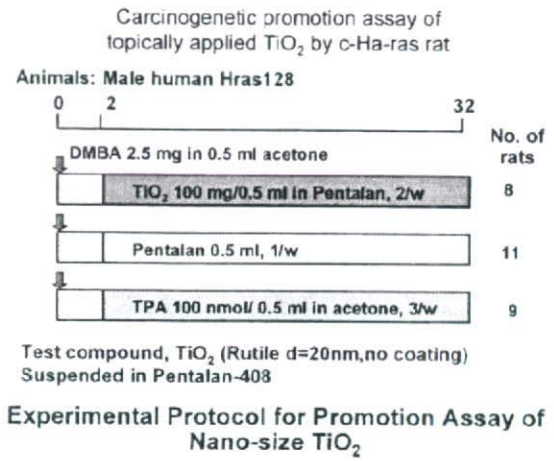

Fig. 6. Carcinogenetic Promotion Assay of Topically Applied TiO_2 by c-Ha-ras Rat

には懸濁溶媒（Pentalan）を用いた．その結果，二酸化チタンは皮膚乳頭腫の発生を促進する結果を得ている（投稿準備中）．

次に，気管内投与による肺発がん促進作用を同様のラットを用いて検討した（Fig. 7）．ジヒドロキシプロピルニトロサミン（DHPN）を2週間飲水投与したのち，隔週で二酸化チタンの懸濁液を気管内スプレーにて，12週間投与した．その結果，肺の過形成病変（前駆病変と考えられる）及び肺腺腫の発がん促進が認められた（投稿準備中）．フラーレンについても同様の実験が進行中である．

最後に，p53ヘテロ欠失マウスの腹腔内投与モデルを用いてのMWCNTのアスベスト様中皮腫発がん性を検討した実験を示す．陽性対象には青アスベスト（Crocidolite）を用いた．フラーレンを非ファイバー状炭素系マテリアルとしての対照に置いた（Fig. 8）．まず，腹腔内投与法の位置付けであるが，アスベスト及び代替繊維，特にグラスファイバーを用いた1970–80年代頃の多くの研究[2-4]の結果からFig. 9に示すごとく，2005年のWHO会合[5]においてもその有効性が認められている．われわれが毒性影響を検討しているMWCNTは，前述のごとく，5 μmよりも長い繊維状あるいは棒状の粒子を含んでいる．これは，もしも十分に頑丈で生体内に長期に渡り分解されずに残留した場合，1978年のPott[6]の「繊維の発がん因子」に当てはめると，Fig. 10の上段左の灰色で示した長方形の領域に当たる繊維をこのMWCNTが含んでいることが分り，当然の帰結として，アスベスト様発がん作用

を有していることが予測される．上段右に示すように，アスベストのp53ヘテロ欠失マウスの腹腔内投与は，野生型マウスにおける中皮腫発がんの期間を短縮することがKaneらのグループにより示されている．[7][8]

用量の設定については，M. Rollerらの論文[9]にあるように（Fig. 11）中皮腫発がん性の弱い繊維の検出が可能な腹腔内投与量が10^9～10^{10}本であることを参考に設定した．この本数で陰性であれば問題ないことになるとの考えである．この実験の結果はFig. 12に示すように，MWCNTが青アスベストと同等の中皮腫発がん性を持つことが示された[10]．なお，体内滞留時間が長いチタン酸カリウムウィスカー，炭化ケイ素ウィスカー，酸化チタンウィスカーはいずれもラット腹腔内投与による中皮腫発がんが知られている．[11]

ナノマテリアルの有害性評価は，ヒトへの大掛かりな暴露が始まっていない現在，製造者側への安全な製品開発に必要な情報提供としての意味合いが大

Asbestos-like fiber carcinogenesis assay of MWCNT
by intraperitoneal administration to p53+/- mouse

Experimental Design

・animal	p53+/- mouse (C57BL/6 back, 9 - 11weeks age)	
	4 groups (18 - 19 per group)	
・administration	single intraperitoneal administration	
	1. MWCNT	3mg/animal = 1x10⁹ f/animal
	2. fullerene	3mg/animal
	3. crocidolite	3mg/animal = 1x10¹⁰ f/animal
	4. vehicle	
・sample preperation	suspended in 0.5%CMC solution, autoclaved, added tween 80 (1%), and sonicated	

Fig. 8. Asbestos-like Fiber Carcinogenesis Assay of MWCNT by Intraperitoneal Administration to p53+/− Mouse

WHO Workshop on Mechanisms of Fibre Carcinogenesis and Assessment of Chrysotile Asbestos Substitutes
8-12 November 2005, Lyon, France "SUMMARY CONSENSUS REPORT"

- **Methodological Aspects**

Epidemiologic studies
a clear advantage, but does not always override contrary findings from toxicological studies.
In in vivo animal studies,
- carcinogenic response (lung cancer, mesothelioma) and fibrosis were considered to be the key effects;
- epithelial cell proliferation; and inflammation were not regarded to be equally important indicators
- the sensitivity of lung tumors in the rat is clearly lower than that in humans. (reason is still unclear)
- **testing of fibres by intraperitoneal injection represents a useful and sensitive assay.**

Fig. 9. WHO Workshop on Mechanisms of Fibre Carcinogenesis and Assessment of Chrysotile Asbestos Substitutes

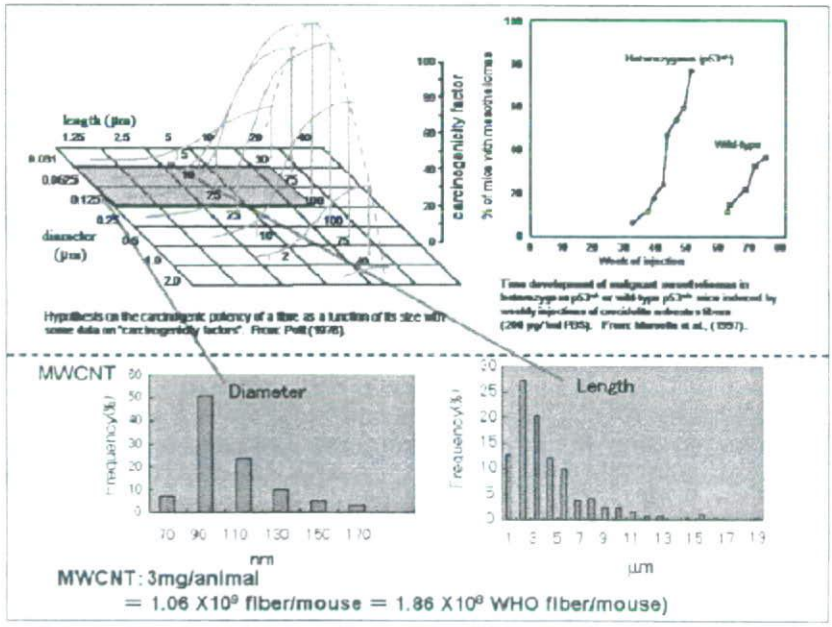

Fig. 10. MWCNT, Its Size and Property

きい．今までの新素材の中には，慢性毒性情報の乏しいことが「低毒性」であることと誤解され，数十年の歳月を掛けて国民的人体実験の結果として中皮腫発がんが顕在化した例がある．国を挙げての新技術開発を担うナノマテリアルは，少なくとも既知の科学的根拠に依拠した有害性によって未来に汚点を残すことだけは，その中長期的経済発展の観点からも，国民の安全の立場からも，避けなければならない．

MWCNT の中皮腫誘発性の問題は，これから検討されるべきナノサイズの粒子の有害性同定（毒性評価）とは別のものである[12-17]．言い換えると，本当のナノサイズの粒子そのものの有害性同定は始まったばかりであり，毒性学の今後の大きな課題である．細胞内の酵素などのタンパク質分子とほとんど同じサイズのナノマテリアルの毒性メカニズムは，今までの毒性学の常識では同定・解析できない可能性もある．最先端の分子毒性学との連携が必須の要因となることが想定される．今後の安全性確保体制の強化には，不足する毒性学研究者の補充と育成も急務である．

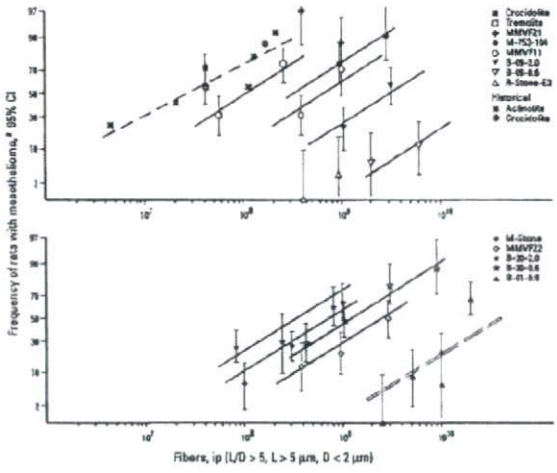

Fig. 11. Fiber Mesotheliomagenesis, Comparative Data among Various Fibrous Amterials

REFERENCES

1) Park C. B., Fukamachi K., Takasuka N., Han B. S., Kim C. K., Hamaguchi T, Fujita K, Ueda S, Tsuda H., *Cancer Sci. Mar.*, **95**(3): 205–210 (2004).
2) World Health Organization "Environmental Health Criteria 53. Asbestos and Other Natural Mineral Fibres." World Health Organization, Geneva (1986).
3) World Health Organization "Environmental Health Criteria 203. Chrysotile Asbestos." World Health Organization, Geneva (1986).
4) World Health Organization "Man-made vitreous fibres. IARC monographs on the evaluation of carcinogenic risk to humans.," Vol. 81, IARC Lyon (2002).

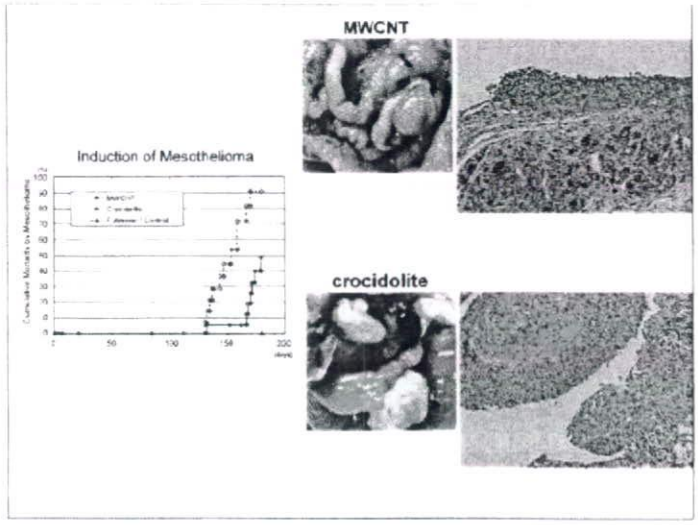

Fig. 12. Induction of Mesothelioma in p53+/− Mouse by Intraperitoneal Application of Multi-wall Carbon Nanotube

5) http://www.who.int/ipcs/publications/new_issues/summary_report.pdf
6) Pott F., Staub-Reinhalt. Luft, **38**, 486–490 (1978).
7) Marsella J. M., Liu B. L., Vaslet C. A., Kane A. B., *Health Perspect.*, **105** (Suppl. 5), 1069–1072 (1997).
8) Vaslet C. A., Messier N. J., Kane A. B., *Toxicol. Sci.*, **68**, 331–338 (2002).
9) Roller M., Pott F., Kamino K., Althoff G. H., Bellmann, B., *Environ. Health Perspect.*, **105** (Suppl. 5), 1253–1256 (1997).
10) Takagi A, Hirose A, Nishimura T, Fukumori N, Ogata A, Ohashi N, Kitajima S, Kanno J., *J, Toxicol. Sci.*, **33**(1), 105–116 (2008).
11) Adachi S., Kawamura K., Takemoto K., *Ind. Health*, **39**, 168–174 (2001).
12) Gulumian M., van Wyk J. A., *Chem. Biol. Interact.*, **62**, 89–97 (1987).
13) Moalli P. A., MacDonald J. L., Goodglick L. A., Kane A. B., *Am. J. Pathol.*, **128**, 426–445 (1987).
14) Lippmann M., *Environ. Health Perspect.*, **88**, 311–317 (1990).
15) Pott F., Roller M., Kamino K., Bellmann B., *Environ. Health Perspect.*, **102**, (Suppl. 5), 145–150 (1994).
16) Bernstein D. M., Riego Sintes J. M., *European Chemicals Bureau*, 44–45 (1999).
17) Hei T. K., Xu A., Huang S. X., Zhao Y., *Inhal. Toxicol.*, **18**, 985–990 (2006).

Trends in Cancer Research
Vol. 3, 2007

Review

Rat mammary preneoplasia and neoplasia: a model for human breast cancer research

Yoichiro Matsuoka[1,*], Hiroaki Kawaguchi[2], Hiroki Yoshida[2], Hiroyuki Tsuda[3] and Airo Tsubura[1]

[1]Second Department of Pathology, Kansai Medical University, 10-15 Fumizono-cho, Moriguchi, Osaka 570-8506, Japan. [2]Department of Tumor Pathology, Field of Oncology, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan. [3]Department of Molecular Toxicology, Nagoya City University Graduate School of Medical Sciences, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan

ABSTRACT

An experimental system that biologically and histologically mimics human breast cancer is needed to understand the pathogenesis of the disease and to build strategies for its prevention and cure. The experimental system should be a tool to assess the influence of host factors, such as age, reproductive history, and genetic background, as well as environmental factors. Rat mammary gland carcinogenesis is the model that most closely fulfills these conditions.

KEYWORDS: carcinogenesis, experimental model, mammary carcinoma, preneoplasia, rat

INTRODUCTION

Breast cancer remains the most frequent type of cancer in women worldwide. The incidence of breast cancer in Western countries is leveling off and may have recently started to decline, especially in women younger than 40 years [1]. However, the incidence of this disease is definitely increasing throughout Asia.

A possible genetic contribution to breast cancer risk is indicated by the increased incidence of breast cancer among women with a family history of breast cancer and by the observation of families in which multiple family members develop breast cancer. Other risk factors for breast cancer include: age, previous breast disease, reproductive and menstrual history, estrogen therapy, radiation exposure, diet, and alcohol intake. While many epidemiological studies have addressed the effects of these risk factors in women, an experimental system that closely mimics human breast cancer is needed to directly investigate the factors that contribute to breast cancer risk. The rat mammary gland treated with carcinogens is one of the most widely studied and useful models of mammary carcinogenesis. In this review, we describe the development of rat mammary glands, induction and pathogenesis of the mammary tumors, and utility of the induced tumors for human breast cancer research.

*Corresponding author
matsuoyo@takii.kmu.ac.jp

Development of rat mammary gland

The proliferation and differentiation of the mammary gland involves a variety of hormones and growth factors, such as estrogen, progesterone, prolactin, hepatocyte growth factor, growth hormone, and IGF-1 [2-4]. The functional and structural development of the gland itself can be divided into seven stages: embryonic, postnatal, juvenile, puberty, pregnancy, lactation, and involution.

Embryonic stage

By day 11 of gestation, two parallel ridges of ectoderm lateral to the midline extend from the

shoulder to the inguinal region, forming the mammary streaks in the fetal rat. Downgrowths of ectodermal epithelium from the mammary streaks form the 12 primary buds from which the 6 pairs of mammary glands develop. There is one pair located in the cervical region, two pairs in the thoracic region, one pair in the abdominal region, and two pairs in the inguinal region [5].

Postnatal stage

The major development of the mammary gland in the rat occurs between birth and puberty. By the end of the first week, each mammary gland consists of a single primary or main lactiferous duct with three to five secondary ducts. During the second week, the secondary ducts branch dichotomously into third, fourth, and fifth generations of ducts.

Juvenile stage

Starting at about the fourth week, growth of the mammary ducts increases significantly. The growth rate of the gland now exceeds the previous isometric rate. Ductal arborization is initiated from the highly proliferative terminal end buds (TEBs) found at the tips of the ductal branches [6] (Figure 1), and ductal morphogenesis and lumen formation is accomplished by a highly regulated process of cell proliferation and cell death [7]. The number of TEBs reaches a maximum at 20 days of age and then decreases as the TEBs differentiate into terminal ductules and alveolar buds (ABs) [5]. TEBs are influenced by systemic steroid hormones and aid the ducts in linear growth as well as the regulation of branching patterns. The ductal pattern is created by the penetration of TEBs through the stromal fat pad. The TEB consists of two histologically distinct cell types. The body cells give rise to mammary epithelial cells while the cap cells are the precursors of the myoepithelial cells [7].

Puberty

Puberty, defined as the onset of estrous cycles, commences in the rat between 35 and 42 days of age. At the onset of puberty, each AB develops into 10 to 12 alveoli to form a lobule, which accumulates progressively over multiple estrous cycles [5, 8]. By 85 days of age, virgin female rats have a relatively constant number and proportion of TEBs, ABs, and lobules, although histological changes in size and secretory development occur within the lobules during the estrous cycle [9-11].

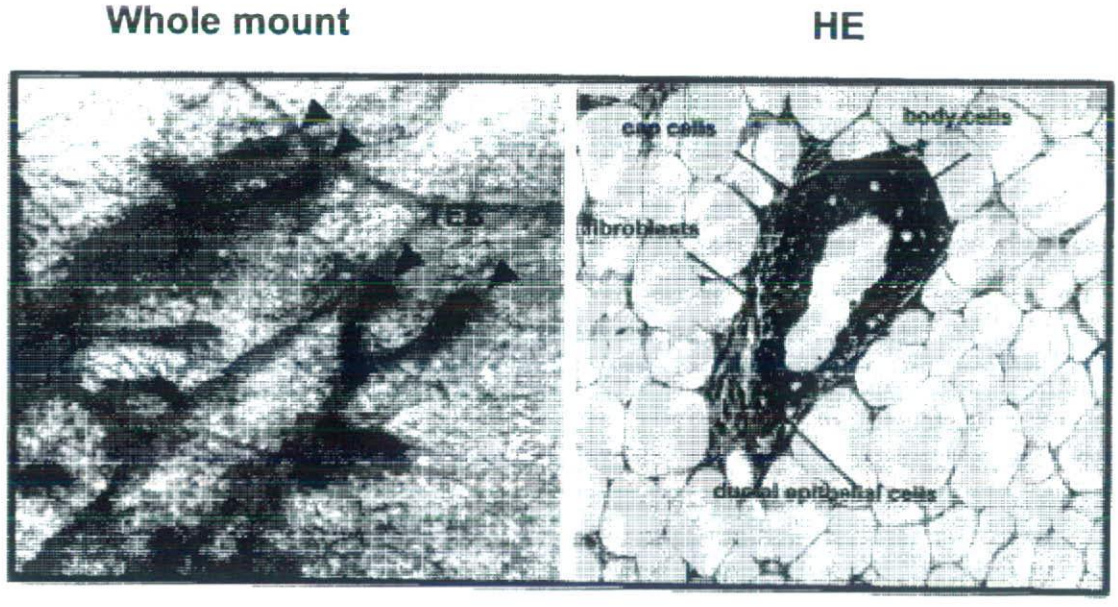

Figure 1. Terminal end buds
Whole mount preparation (left) and hematoxylin-eosin (HE) staining (right) of 49-day-old virgin rat mammary gland showing terminal end buds (TEBs).

Cellular structures of mature mammary gland

The mammary gland can be divided into two compartments: the epithelial (or parenchymal) compartment and the stromal (or mesenchymal) compartment. At the cellular level, the parenchymal compartment of the mammary gland is composed of two different types of epithelial cells with distinct morphologies, functions, and proliferative activities. These cell types are the luminal epithelial cells, which are located along ducts, ductules, terminal ducts, and alveoli, and the myoepithelial cells.

The luminal epithelial cells are cuboidal or columnar in shape [12], and can be defined immunohistochemically by the expression of keratins 8, 18, and 19 [13, 14]. These cells develop the majority of mammary carcinomas [15].

The myoepithelial cells lie between the luminal epithelial cells and the basement membrane [16-19]. The shape, thickness, and continuity of these cells vary during development and between individual epithelial structures in the mammary glands. The myoepithelial cells synthesize and secrete the continuous basement membrane that separates the epithelium from the stromal compartment [12, 20]. The myoepithelial cells express higher levels of cell adhesion receptors and adhesion-associated molecules than the luminal epithelial cells [19]. The former cells can be immunohistochemically distinguished from the latter cells by the expression of intermediate filament proteins (vimentin, keratin 5, and keratin 14) and contractile proteins (myosin and smooth muscle actin) [13, 14, 21].

Pregnant stage

During pregnancy, there is a rapid and continuous increase in the mammary gland epithelium resulting in growth of the lobules and the ducts [12, 22]. The alveoli develop and increase in size and number until the space between ducts is almost completely filled with them. The number of alveoli per unit area shows a large increase from day 5 to day 10 of pregnancy and peaks at day 20 of gestation [12]. During pregnancy, the mammary gland is influenced by estrogen, progesterone, and other placental hormones. The duration of pregnancy is usually 21 days in the rat.

Lactational stage

Lactation is the production and secretion of milk. The initiation of lactation appears to be induced by a decrease in estrogen and progesterone. About 20% of total mammary growth occurs during the first 14 days of lactation. Several hormones, such as prolactin, insulin, and glucocorticoid, are involved in the maintenance of lactation.

Involution

After weaning, there is involution of the glands with a three-fold reduction in the size of lobules; however, the number of lobules remains high, and the gland never returns to the same level of differentiation as seen in virgin female rats of the same age [5]. Involution has been attributed to several factors, including falling levels of circulating prolactin upon the cessation of suckling, mild ischemia as a result of milk engorgement and compression of the vasculature, factors in milk that promote cell death, physical distension of the luminal epithelium, and increased activity of basement membrane-degrading enzymes [12]. Mammary involution comprises two distinct phases. The first phase is apoptosis among the secretory epithelial cells. The second phase is characterized by the degradation of the alveolar structures and the mammary basement membrane and extracellular matrix [23-27]. The apoptosis-induced signals and the loss of survival factors may exert significant control over mammary gland involution.

Induction of mammary lesions in rats

While many strains of rats develop spontaneous tumors later in life, they respond to chemical carcinogens and radiation with faster development of both hormone-dependent and hormone-independent mammary tumors. For the specific induction of mammary tumors in rats via genotoxic mechanisms, the most commonly used agents are 7,12-dimethylbenz[a]anthracene (DMBA) and N-methyl-N-nitrosourea (NMU). A single dose of DMBA (2.5-20 mg) or NMU (25 or 50 mg/kg body weight) by gavage or by intravenous or subcutaneous routes, respectively, induces mammary tumors with latencies of 8 to 21 weeks [28, 29]. Other chemicals such as benzo[a]pyrene, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 1,2-dibromo-3-chloropropane can induce mammary carcinomas with lower incidences [5, 30, 31] (Table 1). Sublethal doses of different types of radiation, including x-rays and neutrons,

Table 1. Induction of mammary tumors by chemical or physical agents.

Carcinogen	Lesion Type
Genotoxic Agents	
DMBA	Ductal / Alveolar/ Mesenchymal?
NMU	Ductal / Mesenchymal?
benzo[a]pyrene[30]	Ductal
2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP)	Ductal / Mesenchymal
1,2-Dibromo-3-chloropropane[116]	Ductal
1,2-Dibromoethane[117]	Alveolar/ Mesenchymal?
Ochratoxin A[118]	Alveolar/ Mesenchymal?
Radiation (x-rays, γ-rays, neutrons)	Ductal / Mesenchymal
Non- Genotoxic Agents	
Estrogens[119, 120]	Ductal

induce mammary tumors within a year [32, 33] (Table 1). Induced tumors are histologically benign and malignant, and they generally have features in common with human tumors.

Chemically-induced mammary preneoplastic and neoplastic lesions

With chemical carcinogens, the earliest visible histological changes in the rat mammary glands are focal or multifocal hyperplasias primarily within the terminal ductule or AB or both [34-36]. The TEBs and terminal ducts are the sites of origin of malignancies, whereas benign lesions such as cysts, adenomas, alveolar hyperplasias, and fibroadenomas originate from the ABs in the rat mammary glands [35-39]. These observations indicate that there are two different pathogenetic pathways: one pathway for malignant lesions and another pathway for benign lesions. In addition, benign lesions tend to appear later than the malignant ones, indicating that the former are not precursors of the latter [35, 40].

Ductal hyperplasia pathway

A common type of chemically-induced preneoplastic lesion found in rats is ductal hyperplasia, which is characterized by intraluminal proliferation of epithelial cells (i.e., an increase in the number of epithelial cell layers within a duct). Ductal hyperplasia, which exhibits intraductal epithelial proliferation, progresses through a phenotype very similar to human ductal carcinoma in situ. In histological sections, intraductal proliferation becomes larger (Figure 2) and leads to the formation of micro-adenocarcinomas. When young virgin rats (21 to 50 days old) are inoculated with DMBA or NMU, ductal hyperplasia is detectable within 14 days after inoculation, and intraductal carcinomas are detectable after 20 days. Locally invasive carcinomas develop from these intraductal lesions to form palpable tumors approximately 13 weeks after the injection [28, 36].

In contrast to conventional strains, a rat strain carrying the human c-Ha-ras protooncogene is highly susceptible to mammary chemical carcinogens [41, 42]. When the transgenic rats are intravenously injected with 50 mg/kg body weight of NMU at 50 days of age, atypical ductal hyperplasias develop in 44% of the animals by day 15, and small invasive carcinomas form in almost all animals by day 20. Adenocarcinomas become palpable in all animals by day 56 [43]. This rat model can be used for short-term screening of chemopreventive agents, as well as mid-term screening of promoting agents for mammary carcinogenesis.

Studies of the pathogenesis of rat mammary carcinomas have revealed that carcinogens act on TEBs and terminal ducts mainly when these structures are differentiating into ABs. Transformed TEBs and terminal ducts evolve into ductal hyperplasias, carcinomas in situ, and invasive carcinomas [35, 38, 44]. In humans, ductal hyperplasias with or without atypia and

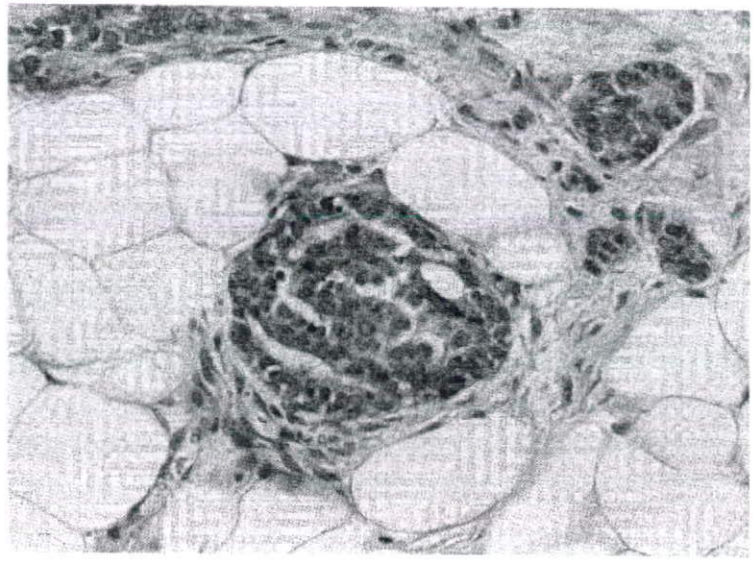

Figure 2. Intraductal epithelial hyperplasia
An enlarged terminal ductal structure exhibiting intraductal proliferations of epithelium.

atypical lobular hyperplasia are considered to be risk factors for subsequent development of invasive breast carcinomas [45-47]. However, the comparison between the pathogenetic pathways of mammary carcinogenesis in rats and humans is only tentative, because the role of the TEBs in humans is unknown. The TEB in the human female is a prepubertal structure, and the biology and differentiation of this structure from prepuberty to puberty needs to be studied. An important difference between the pathogenetic pathways in rats and humans is at the level of the terminal ductal lobular unit. The TEB in the rat would be equivalent to the intralobular terminal duct in the human, the area which is most susceptible to neoplastic growth [28]. Observations of early carcinomas in the human breast are needed to facilitate a clearer understanding of the pathogenetic scheme.

Alveolar hyperplasia pathway

Alveolar hyperplasia is a term commonly applied to enlarged lobules consisting of relatively normal alveoli that resemble the normal prelactating mammary gland [28, 37]. However, minimal or mild degrees of alveolar hyperplasia are difficult to distinguish from the normal state. In rats, the cause and biological behavior of alveolar hyperplasia are unknown, but the lesion is thought to be a precursor of adenoma and/or fibroadenoma (Figure 3). Chemically-induced alveolar hyperplasia does not appear to give rise to adenocarcinomas in rats. By clear contrast, the principal preneoplastic lesion in mouse mammary glands is alveolar hyperplasia or hyperplastic alveolar nodule [48, 49], which can be induced by mouse mammary tumor viruses [50-52], chemical carcinogens [53], X-irradiation [54], and prolonged hormone stimulation [55]. Also, in humans, atypical lobular hyperplasia is thought to progress to invasive lobular carcinoma via lobular carcinoma in situ.

Histopathology of neoplastic lesions

Rat mammary tumors have been classified by several authors [28, 38, 56, 57], and benign tumors, such as intraductal papilloma, papillary cystoadenoma, adenoma, and malignant tumors, such as papillary carcinoma, cribriform carcinoma (Figure 4), comedo carcinoma, and tubular carcinoma, have been recognized. Most of the neoplastic lesions found in the rat mammary glands have their counterpart in human pathology, with the exceptions of human-specific lesions such as lobular carcinoma and Paget's disease. Although lobular carcinoma, in situ or invasive, has not been described in the conventional strains of rats [44], the alveolar epithelia can transform to give rise to carcinomas under a certain genetic

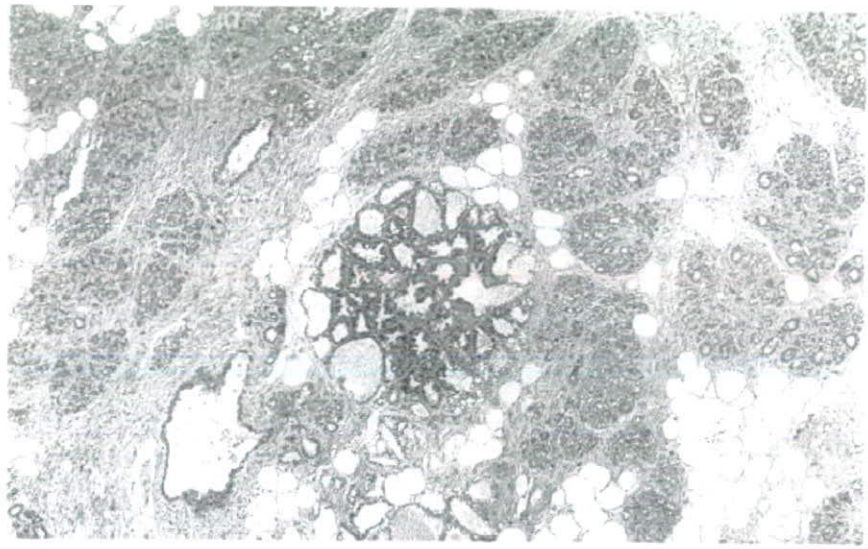

Figure 3. Fibroadenoma with alveolar hyperplasia
Note the proliferation of epithelium resembling alveolar buds and also the papillary growth pattern.

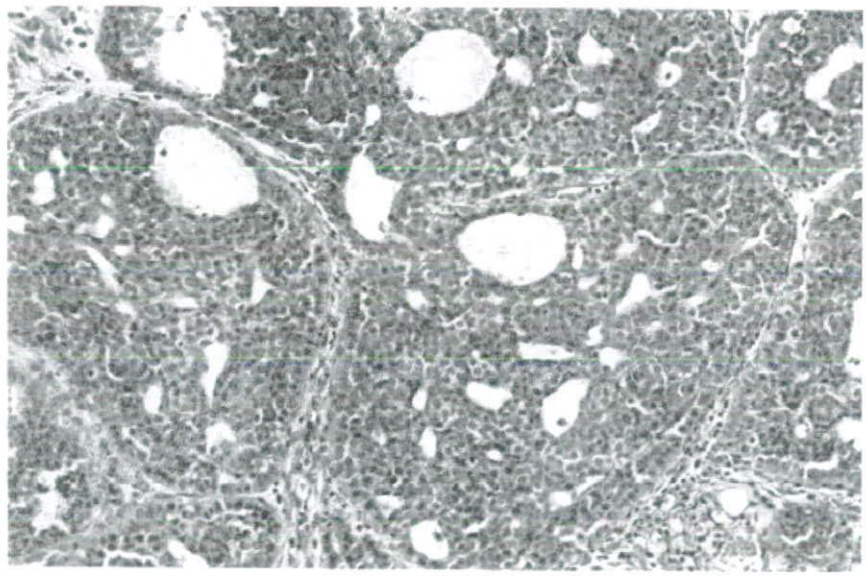

Figure 4. Cribriform carcinoma
Ductal carcinoma with areas of cribriform pattern.

background such as human c-Ha-ras transgenic rats [39] (Figure 5).

Molecular pathology of neoplastic lesions

Mutation analyses of oncogenes and tumor suppressor genes in sporadic human breast cancer and chemically-induced rat mammary carcinomas have revealed both similarities and differences in the mutation spectra of the two types of tumors. Ha-ras mutations are commonly observed with an incidence of 18% to 80% in the rat carcinomas induced by DMBA, NMU, or 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), whereas mutations in Ki-ras, p53, and brca1 are rarely

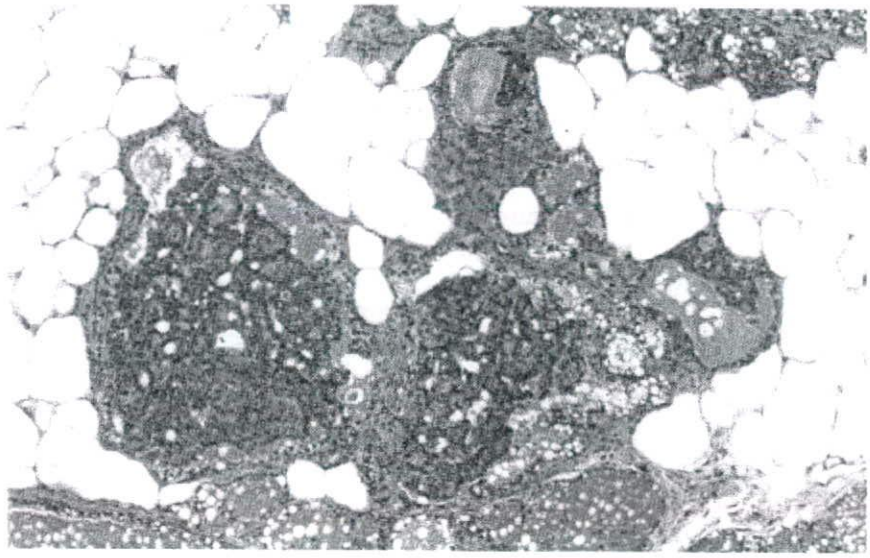

Figure 5. Alveolar hyperplasia with cellular atypia in c-Ha-ras proto-oncogene transgenic rat A marked increase in the number of mammary terminal branches and alveoli in 35-week-old transgenic virgin rats is described in Hamaguchi *et al.* [39]. Occasionally, the epithelial cells of these pre-neoplastic lesions displayed atypia.

Table 2. Genetic alterations in chemically induced rat mammary carcinomas.

	DMBA	NMU	PhIP
Ha-ras	23%[121, 122]	29-80%[41, 121-123]	18-42%[31, 124]
Ki- ras	—	18%[123]	0%[31]
p53	3% [122]	0%[122]	0-10%[31, 125, 126]
brca 1	—	—	0%[127]

detected (Table 2). In contrast, approximately 30% of sporadic human breast cancers have mutations in TP53 (human p53 gene) [58-60], but few human cancers feature Ha-ras or Ki-ras alterations [61, 62]. Amplification and/or overexpression of Ha-, Ki-, and N-ras, cyclin D1, and neu/erbB-2/HER-2 are common in breast malignancies in humans [62, 63]. Two laboratories have demonstrated that overexpression of Ha-ras is sufficient to induce mammary carcinomas in rats [64, Ueda and Tsuda, unpublished data]. Thus, aberrant expression of the ras, cyclin D1, or erbB-2 genes can initiate mammary carcinogenesis in rats as well as in humans.

In both rats and humans there is strong evidence of genetic predisposition. Hereditary breast cancer is characterized by early age at onset (an average of 5 to 15 years earlier than sporadic cases), bilaterality, vertical transmission through both maternal and paternal lines, and familial association with tumors of other organs, particularly the ovary and prostate gland. The search for genes associated with hereditary susceptibility to breast cancer has led to the identification of several susceptibility genes, including BRCA1, BRCA2, TP53, and PTEN/MMAC1. Mutations in the BRCA1 or BRCA2 genes confer a lifetime risk of breast cancer of between 60% and 85% [65, 66]. However, mutations in these genes account for only 2% to 3% of all human breast cancers [67, 68], and susceptibility alleles in TP53 and PTEN/MMAC1 are even less common causes of human breast cancer [69]. The 1100delC mutation

in the cell-cycle-checkpoint kinase gene (CHEK2 or CHK2) has been discovered as an additional gene variant conferring susceptibility to breast cancer [70]. CHEK2 protein is implicated in DNA repair processes involving BRCA1 and p53 [71-73]. While the 1100delC mutation, a truncating variant that abrogates the kinase activity, has a frequency of 1.1% in healthy individuals this variant is present in 5.1% of individuals with breast cancer from 718 families that do not carry mutations in BRCA1 or BRCA2. This mutation doubles the risk of breast cancer among women and increases the risk among men by a factor of 10 [70]. The CHEK2 protein is activated after phosphorylation by the checkpoint gene product ATM and in turn activates BRCA1. The role of ATM mutations in the predisposition to the early onset of breast cancer remains controversial, but some missense mutations appear to increase susceptibility to breast cancer in humans [74] and mice [75]. There is also convincing evidence of the existence of additional high-penetrance genes that increase susceptibility to breast cancer [69].

In human lesions, ductal carcinoma in situ shows multiple losses involving loci on chromosomes 2q, 13q, 16q, 17p, and 17q, which also happen to be sites where important tumor suppressor genes are located [76]. A few studies have been conducted in lobular neoplasia and show losses at 11q, 16q, and 17q in these lesions [47]. Specific genomic alterations have been described in rat mammary carcinomas induced by DMBA, NMU, and PhIP [77, 78]. However, the tumorigenic potential of each of these alterations mostly remains to be tested in rat models.

Risk assessment using the rat carcinogenesis model

Reproductive history is the strongest and most consistent risk factor outside of genetic background and age [79, 80]. Especially, early age of first full-term pregnancy (≦20 years old) is a strong protective factor. On the other hand, a history of induced abortion appears to have little influence on the breast cancer risk [81, 82]. A collaborative re-analysis of data shows that the relative risk of breast cancer decreases by 4.3% for each year that women breastfeed [83]. Protection by parity from mammary carcinogenesis is also observed in rats [84-87], and short-term exposure to pregnancy levels of estrogen is sufficient to this effect and equally protective for even nulliparous rats [88]. Whereas pregnancy alone has been as effective as pregnancy and lactation in most experiments, Yang *et al.* [87] have reported that pregnancy followed by lactation has an additive effect in protection when rats are exposed to NMU prior to pregnancy. Interrupted pregnancy appears to be protective with lower efficiency compared to full-term pregnancy, although the interruption experiments have yielded contradictory results [86, 89, 90].

The age-adjusted death rates from breast cancer are 2 to 8-fold less in Asian countries than in the United States and Western Europe. The smaller death rate appears to be related to the 20- to 50-times greater consumption of soybean products [91]. A case-control study in Shanghai suggested that regular soy consumption reduced the risk of hormone-receptor-positive tumors [92]. Studies using the rat model as well as carcinoma cell lines have indicated that genistein, one of the isoflavones in soybean, may be responsible for tumor suppressive effects [43, 93-96]. A diet rich in folate and carotenoids might also be protective [97-99]. The possibility of a protective role for folate is somewhat controversial in the rat models since moderate folate deficiency inhibits, whereas dietary folate supplementation does not significantly promote, the progression of NMU-induced mammary neoplastic foci [100, 101]. There are relatively few studies of the effects of carotenoids in the rat models. The majority, but not all, of these studies indicate a protective effect of lycopene-rich tomato carotenoid oleoresin, whereas β-carotene shows no protection against the development of mammary cancer [102-104]. A Canadian case-control study found an association with dioxin-like polychlorinated biphenyls, suggesting that exposure to these substances might increase the risk [105]. In rats, however, inconsistent results have been obtained: 3,3',4,4'-tetrachlorobiphenyl significantly inhibits the tumor growth [106] whereas 2,3,7,8-tetrachlorodibenzo-p-dioxin slightly increases the tumor incidence when neonatal rats are initiated with NMU [107].

Greater consumption of dietary fat (especially n-6 polyunsaturated fatty acids) enhances breast cancer

risk [108, 109]. In the rat model, high levels of dietary fat increase the incidence of chemically-induced mammary carcinomas, and affect the promotion phase but not the initiation phase of the carcinogenesis [5]. Both the quantity and the constituents of fat should be considered as the risk factors. An n-6 polyunsaturated fatty acid, linoleic acid, may be responsible for the promoting activity while n-3 polyunsaturated fatty acids, eicosapentaenoic acid and docosahexaenoic acid, may act inversely [110].

An increase in the multiplicity of breast cancer is seen in long-term neuroleptics users [111]. A variety of drugs, such as reserpine and perphenazine, that decrease hypothalamic dopaminergic activity enhance the development, multiplicity, and growth of chemically-induced mammary carcinomas in rats [5, 112]. This effect is probably mediated by prolactin, since dopaminergic activity is primarily responsible for inhibition of prolactin release from the pituitary.

PROSPECTS

Since rat mammary tumors closely resemble the human counterparts in many aspects, genetically engineered rats may serve as a favorable model for human breast cancer research. A major disadvantage of the rat system is that the gene knockout technique has been unavailable. In contrast, almost 100 transgenes, targeted mutations, combinations of transgenes, and combinations of transgenes and targeted mutations have been used to study mammary cancer in mice. Genetically engineered mice tumors have: (1) phenotypes similar to those of non-genetically engineered mice tumors; (2) signature phenotypes specific to the transgene; and (3) some morphological similarities to human breast cancer [113, 114]. However, some investigators did not appreciate the relevance of the murine systems because mouse mammary tumors do not resemble most human breast cancers either morphologically or biologically. Nevertheless, the emergence of knockout and transgenic biologies has provided remarkable evidence that mouse tumors can be produced by the same genes implicated in human breast cancer [115]. The development of the gene knockout technique in rats will be a powerful tool in breast cancer research.

ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-aid for Scientific Research on Priority Areas (KAKENHI) from the Ministry of Education, Science, Sports, and Culture of Japan, a Grant-in-aid for the Second-term Comprehensive 10-Year Strategy for Cancer Control, a Grant-in-aid for Cancer Research, a Health and Labor Science Research Grant for Research on Risk of Chemical Substances from the Ministry of Health, Labour and Welfare of Japan, and by the Nakayama Foundation for Human Science.

REFERENCES

1. Lacey, J. V., Jr., Devesa, S. S., and Brinton, L. A. 2002, Environ. Mol. Mutagen, 39, 82.
2. Rosen, J. M., Humphreys, R., Krnacik, S., Juo, P., and Raught, B. 1994, Prog. Clin. Biol. Res., 387, 95.
3. Kleinberg, D. L. 1998, Breast Cancer Res. Treat., 47, 201-208.
4. Soriano, J. V., Pepper, M. S., Orci, L.. and Montesano, R. 1998, J. Mammary Gland Biol. Neoplasia, 3, 133-150.
5. Boorman, G. A., Wilson, J. T., van Zwieten, M. J., and Eustis, S. L. 1990, Pathology of the Fisher rat, G. A. Boorman, S. L. Eustis, M. R. Elwell, C. A. Montgomery, Jr. and W. F. MacKenzie (Eds.), Academic Press, San Diego, 295.
6. Daniel, C. W., and G. B. Silberstein. 1987, The mammary gland: Development, regulation, and function, M. C. Neville and C. W. Daniel (Eds.), Plenum Press, NewYork, 3.
7. Humphreys, R. C., Krajewska, M., Krnacik, S., Jaeger, R., Weiher, H., Krajewski, S., Reed, J. C., and Rosen, J. M. 1996, Development, 122, 4013.
8. Russo, I. H., and Russo, J. 1978, J. Natl. Cancer Inst., 61, 1439.
9. Dulbecco, R., Henahan, M., and Armstrong, B. 1982, Proc. Natl. Acad. Sci. USA, 79, 7346.
10. Ormerod, E. J., and Rudland, P. S. 1984, Am. J. Anat., 170, 631.
11. Schedin, P., Mitrenga, T., and Kaeck, M. 2000, J. Mammary Gland Biol. Neoplasia, 5, 211.

12. Masso-Welch, P. A., Darcy, K. M., Stangle-Castor, N. C., and Ip, M. M. 2000, J. Mammary Gland Biol. Neoplasia, 5, 165.
13. Dundas, S. R., Ormerod, M. G., Gusterson, B. A., and O'Hare, M. J. 1991, J. Cell Sci., 100 (Pt 3), 459.
14. Bocker, W., Bier, B., Freytag, G., Brommelkamp, B., Jarasch, E. D., Edel, G., Dockhorn-Dworniczak, B., and Schmid, K. W. 1992, Virchows Arch. A Pathol. Anat. Histopathol., 421, 315.
15. Nandi, S., Guzman, R. C., and Yang, J. 1995, Proc. Natl. Acad. Sci. USA, 92, 3650.
16. Moore, D. M., Vogl, A. W., Baimbridge, K., and Emerman, J. T. 1987, J. Cell. Sci., 88, (Pt 5), 563.
17. Koukoulis, G. K., Virtanen, I., Korhonen, M., Laitinen, L., Quaranta, V., and Gould, V. E. 1991, Am. J. Pathol., 139, 787.
18. Kim, N. D., and Clifton, K. H. 1993, Exp. Cell Res., 207, 74.
19. Glukhova, M., Koteliansky, V., Sastre, X., and Thiery, J. P. 1995, Am. J. Pathol., 146, 706.
20. Gordon, J. R., and Bernfield, M. R. 1980, Dev. Biol., 74, 118.
21. Dulbecco, R., Unger, M., Armstrong, B., Bowman, M., and Syka, P. 1983, Proc. Natl. Acad. Sci. USA, 80, 1033.
22. Joshi, K., Ellis, J. T., Hughes, C. M., Monaghan, P., and Neville, A. M. 1986, Lab. Invest., 54, 52.
23. Quarrie, L. H., Addey, C. V., and Wilde, C. J. 1995, Cell Tissue Res., 281, 413.
24. Quarrie, L. H., Addey, C. V., and Wilde, C. J. 1996, J. Cell Physiol., 168, 559.
25. Lund, L. R., Romer, J., Thomasset, N., Solberg, H., Pyke, C., Bissell, M. J., Dano, K., and Werb, Z. 1996, Development, 122, 181.
26. Streuli, C. H., and Gilmore, A. P. 1999, J. Mammary Gland Biol. Neoplasia, 4, 183.
27. Jerry, D. J., Pinkas, J., Kuperwasser, C., Dickinson, E. S., and Naber, S. P. 1999, J. Mammary Gland Biol. Neoplasia, 4, 177.
28. Russo, J., Gusterson, B. A., Rogers, A. E., Russo, I. H., Wellings, S. R., and van Zwieten, M. J. 1990, Lab. Invest, 62, 244.
29. Thompson, H. J., and Adlakha, H. 1991, Cancer Res., 51, 3411.
30. Rees, E. D., Shuck, A. E., Lowry, J. Q., Smith, T. M., and Lipscomb, H. 1979, J. Environ. Pathol. Toxicol., 2, 1475.
31. Ushijima, T., Kakiuchi, H., Makino, H., Hasegawa, R., Ishizaka, Y., Hirai, H., Yazaki, Y., Ito, N., Sugimura, T., and Nagao, M. 1994, Mol. Carcinog., 10, 38.
32. Shellabarger, C. J., Bond, V. P., Aponte, G. E., and Cronkite, E. P. 1966, Cancer Res., 26, 509.
33. Broerse, J. J., Hennen, L. A., and Solleveld, H. A. 1986, Leuk. Res., 10, 749.
34. Yoshida, H., Kadota, A., and Fukunishi, R. 1980, Virchows Arch. B Cell Pathol. Incl. Mol. Pathol., 34, 33.
35. Russo, J., and Russo, I. H. 1987, Lab. Invest., 57, 112.
36. Thompson, H. J., McGinley, J. N., Wolfe, P., Singh, M., Steele, V. E., and Kelloff, G. J. 1998, Carcinogenesis, 19, 2181.
37. Russo, J., and Russo, I. H. 1996, Breast Cancer Res. Treat., 39, 7.
38. Singh, M., McGinley, J. N., and Thompson, H. J. 2000, Lab. Invest., 80, 221.
39. Hamaguchi, T., Matsuoka, Y., Kawaguchi, H., Fukamachi, K., Takasuka, N., Ueda, S., Shimizu, K., Ohki, M., Kusunoki, M., Sakakura, T., Yoshida H., and Tsuda, H. 2004, Breast Cancer Res. Treat., 83, 43.
40. Russo, J., Tay, L. K., and Russo, I. H. 1982, Breast Cancer Res. Treat., 2, 5.
41. Asamoto, M., Ochiya, T., Toriyama-Baba, H., Ota, T., Sekiya, T., Terada, M., and Tsuda, H. 2000, Carcinogenesis, 21, 243.
42. Han, B. S., Fukamachi, K., Takasuka, N., Ohnishi, T., Maeda, M., Yamasaki, T., and Tsuda, H. 2002, Carcinogenesis, 23, 1209.
43. Matsuoka, Y., Fukamachi, K., Hamaguchi, T., Toriyama-Baba, H., Kawaguchi, H., Kusunoki, M., Yoshida, H., and Tsuda, H. 2003, Toxicol. Pathol., 31, 632.
44. Russo, J., Russo, I. H., Rogers, A. E., van Zwieten, M. J., and Gusterson, B. 1990, IARC Sci. Publ., 47.
45. Tavassoli, F. A., and Norris, H. J. 1990, Cancer, 65, 518.
46. Dupont, W. D., Parl, F. F., Hartmann, W. H., Brinton, L. A., Winfield, A. C., Worrell, J. A., Schuyler, P. A., and Plummer, W. D. 1993, Cancer, 71, 1258.